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Molecular-cytogenetic detection of a deletion of 1p36.3

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Abstract

We report a deletion of 1p36.3 in a child with microcephaly, mental retardation, broad forehead, deep set eyes, depressed nasal bridge, flat midface, relative prognathism, and abnormal ears. The phenotype is consistent with that described for partial monosomy for 1p36.3. Reverse chromosome painting and microsatellite and Southern blot analyses were used to map the extent of the deletion. Fluorescence in situ hybridisation (FISH) analysis using probes from every telomere indicates that the rearrangement is likely to be a chromosomal truncation or rearrangement involving subtelomeric repetitive DNA. The deletion was identified by screening a sample of children and adults with idiopathic mental retardation. In conjunction with previous work on this sample, we estimate that 7.4% of the group have subtelomeric rearrangements.

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It is often suspected that many cases of idiopathic mental retardation are the result of cytogenetically invisible chromosomal rearrangements. These small chromosomal anomalies can be detected using hypervariable polymorphic probes (HVPs) to determine whether affected children receive two copies of each locus examined in a normal mendelian pattern. However, the very large number of analyses involved in screening the whole genome at a high resolution has prompted attempts to identify chromosomal regions that might be more likely to incur pathogenic rearrangements.

We have recently shown that the frequency of rearrangements at the ends of chromosomes (telomeres) is high in cases of idiopathic mental retardation. In our initial survey of 99 children and their parents, we identified three children (3%) with cryptic chromosomal anomalies involving telomeric regions.[1] The frequency of subtelomeric abnormalities is likely to be higher than 3% because the test used was deficient in two ways. First, only 28 chromosome ends out of a possible 41 (excluding acrocentric p arms) were examined. Second, in some cases parents shared the same sized alleles, so that HVP analysis could not distinguish a homozygote child from a hemizygote. By taking these deficiencies into account we estimated that the frequency of rearrangements in our sample was likely to be at least 6%.[1 2]

We now report the results of analysing our sample with one more HVP from the terminal region of chromosome 1p. We have detected a chromosomal deletion of 1p that was not apparent on routine cytogenetic testing in a child with developmental delay and congenital anomalies. Further analysis with 1p probes, reverse painting, and FISH with telomere specific cosmids suggests that the child has a pure monosomy of the terminal 5-7 megabases (Mb) of 1p.

Materials and methods

DNA ANALYSIS

Venous blood samples were taken from the patient and both parents. Epstein-Barr virus transformed lymphoblast cultures were established (available on request to JF). DNA was extracted from cell lines and peripheral blood by standard procedures.[3] Samples from both parents and the affected offspring were digested with an appropriate restriction endonuclease, electrophoresed through 0.8% agarose gels, transferred to nylon membranes, and hybridised to radioactively labelled probes.[3]

PROBES AND GENETIC MAPPING

Minisatellites used in this report are listed in table 1 and were cloned using the approach previously described[4 5] and unpublished procedures. The cosmid containing CEB108 and the chromosome 1 cosmid for D1F101S2 are derived from the ICRF chromosome 1 cosmid library. All the others are derived from a commercial library.[4]

The minisatellite loci have been genetically mapped by typing of 40 CEPH families and heterozygosity estimated from 80 CEPH parents. The sex average distance from the 1p telomere is deduced from our unpublished data at two more distal minisatellite loci (D1F35S2 and D1F102S1) and computed using CRIMAP version 2.4. All genotyping data are available from CEPH database version 8.1 (http://www.cephb.fr/). The most telomeric AFM microsatellite (D1S243) and the midisatellite D1Z2 are in the CEB15-CEB88 interval.

Microsatellites were purchased from Research Genetics. PCR reactions were performed in a volume of 15 µl with 50 ng of genomic DNA, 6 pmol of each primer, and 0.5 units of thermostable DNA polymerase in a buffer containing 10 mmol/l Tris HCl (pH 8.3), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, and

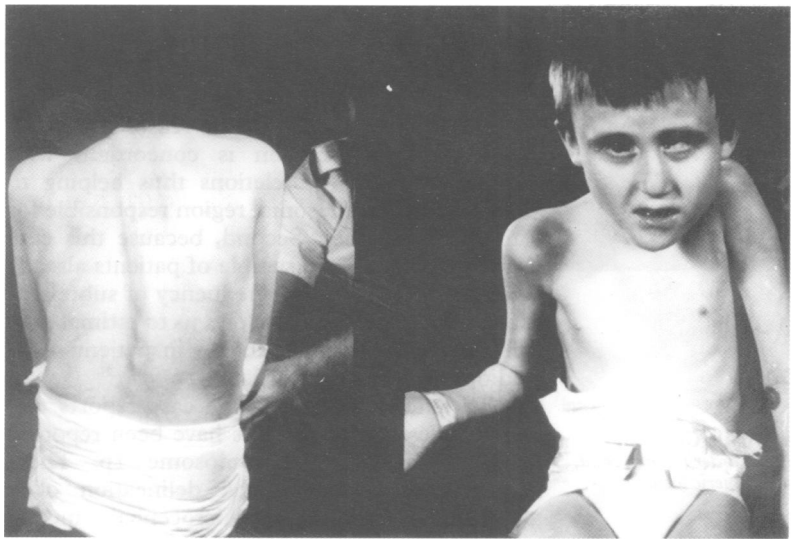

Figure 1 Photographs of the patient at the age of 11. Note the broad forehead, deep set eyes, and skeletal deformities. (Photographs reproduced with permission.)

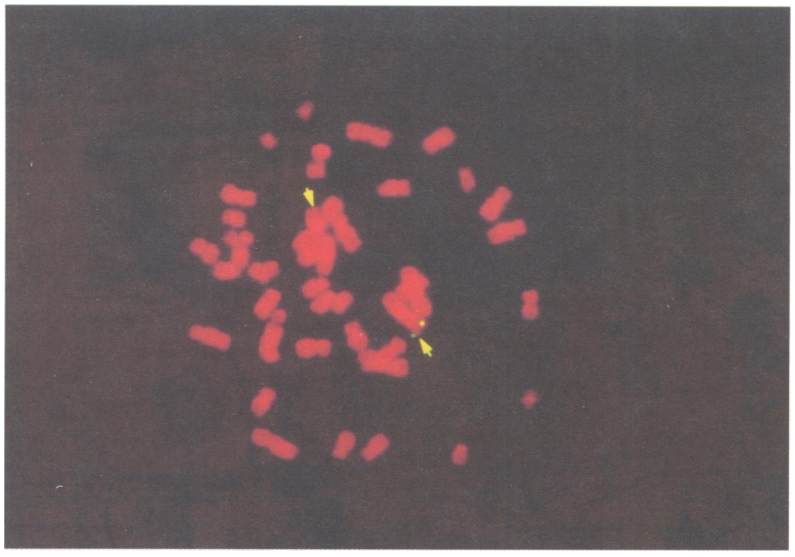

Figure 2 Fluorescence in situ hybridisation of cosmid CEB108 to metaphases of the patient's EBV transformed lymphocytes. Arrows indicate the telomere of chromosome 1p. A fluorescent signal is seen on only one chromosome 1 homologue at the telomere.

100 µmol/l of each dNTP. Thirty amplification cycles were used with an annealing temperature of 55°C. Products were separated on 6% polyacrylamide/urea gels, blotted onto Hybond-N+ (Amersham), and hybridised to $(AC)_{10}$ end labelled with α-32P dCTP using terminal transferase (Boehringer Mannheim). CA repeat loci examined were D1S214, D1S244, D1S228, and D1S234.[6]

CYTOGENETICS

Metaphase chromosomes were prepared from PHA stimulated peripheral blood lymphocytes or EBV transformed B lymphocytes using a thymidine synchronisation method.[7] G banding was performed by trypsin treatment and staining with Leishman's to give a GTG banded pattern.

FLUORESCENCE IN SITU HYBRIDISATION

FISH was performed on lymphoblastoid cells from patient and parents. Cosmids were labelled with biotin-16-dUTP by nick translation, and hybridised according to standard protocols.[7] A total of 100 ng of conjugated probe and 2.5 µg of competitor DNA was used per slide. Probes were detected by fluorescein isothiocyanate conjugated avidin.

REVERSE CHROMOSOME PAINTING

Metaphase chromosomes were sorted on a Becton Dickinson FACStar Plus as previously described.[8] Five hundred chromosomes were amplified with degenerate primers (DOP-PCR) and 5 µl of the product amplified again in the presence of biotin-16-dUTP.[7 8] A total of 100 ng of labelled chromosomes with 6.25 µg of competitor DNA was hybridised to normal male metaphase chromosomes, following the protocol described above for FISH.

Results

CLINICAL REPORT

The patient is the 16 year old son of healthy, non-consanguineous parents with no family history of physical or mental disorder. He was born at term after an uneventful pregnancy, weighing 2490 g (10th centile) and with OFC 33 cm (25th centile). In the neonatal period he was noted to have an unusual facial appearance with a large anterior fontanelle and widely separated sagittal sutures. At 3 months he was admitted to hospital with right sided focal fits. Moderate bilateral optic atrophy was observed when he was examined under anaesthesia. From that time he has continued to have seizures. In addition he has had a progressive neuromuscular scoliosis (no underlying vertebral abnormality has been detected) which required surgical intervention. After spinal fusion with insertion of rods his spine has been relatively stable.

Examination of the patient at the age of 15 years 9 months showed severe retardation. His head circumference was 49.5 cm (<<3rd centile). He had plagiocephaly with a broad flat forehead and hypotelorism, bilateral rotatory nystagmus, a right ear with no lobe that was 5 mm shorter than the left ear, and a small nose with deviation of the nasal septum (fig 1). In addition he had a small mouth, overcrowded dentition, a flat ridged palate, and midface hypoplasia. He had mild syndactyly of the second and third fingers and short thumbs. His feet were thin with short digits and both halluces showed distal valgus deviation. He had a severe scoliosis, small genitalia, and no evidence of puberty.

MOLECULAR CYTOGENETIC ANALYSIS

The sample of 99 children[1] was screened by Southern blot analysis using the probe CEB108. The patient described here had inherited only a single allele at this locus, indicating either a deletion of the maternally derived chromosome, the inheritance of two copies of the paternal chromosome (isodisomy), or a new mutation of the maternal allele to the same size as the paternal allele, making him a homozygote. FISH using CEB108 showed that there was a deletion of 1p (fig 2).

We next attempted to define the nature and extent of the rearrangement. G banded chromosome analysis at the 440-500 band level was normal. FISH analyses of both parents were

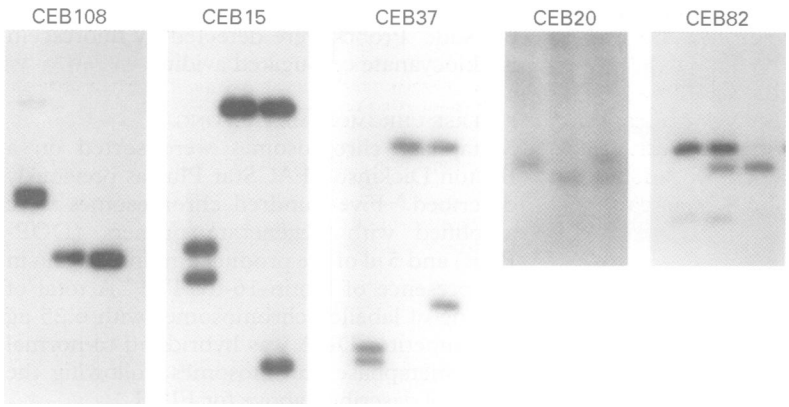

Figure 3 Southern blot analysis of five telomeric 1p probes from cosmids CEB108, CEB15, CEB37, CEB20, and CEB82. DNA samples are run in the order mother, child, father. The child is missing a maternal allele, except at the most centromeric locus (CEB82).

normal indicating that the rearrangement was not inherited (data not shown). We then sorted and labelled the patient's chromosomes 1 and hybridised them to a normal male karyotype. The reverse paint experiment showed signal on chromosome 1 only, suggesting that the patient's abnormal chromosome was either a pure deletion or contained only a small amount of translocated material.[7] In order to detect smaller rearrangements we carried out a series of FISH analyses using clones that, with four exceptions, have been physically linked to within 500 kb of the telomere of each chromosome.[9] Included within this analysis was the bacteriophage clone λ14 that hybridises to all the acrocentric p arms and to 4p.[10] No abnormality was detected in any of these experiments. The data are consistent with a pure deletion of 1p (owing to a chromosome truncation or interstitial deletion into subtelomeric repetitive sequence), but we have not ruled out a translocation involving subtelomeric repetitive DNA.[11]

In order to examine the extent of the deletion we used HVP, FISH, and microsatellite analyses (fig 3, table 1). The order along the chromosome of the probes used, their genetic distance from each other and from the telomere, and their presence or absence on the abnormal chromosome is given in table 1. Table 1 shows that the deletion extends from the most telomeric marker to between D1S214 at 16.4 cM and D1S244 at 22.9 cM. Note that this genetic distance is compatible with a physically small deletion because of the known genetic map expansion that occurs at telomeric regions.[12 13]

Table 1 The hypervariable probes used for Southern blot and FISH analysis (probes prefixed CEB), and microsatellite analysis (markers prefixed AFM). The heterozygosity and genetic distance from the 1p telomere is given for each probe and marker

Locus symbol	*Marker name*	*Het (%)*	*Distance from telomere (cM)*	*HVP analysis*	*FISH analysis*
ND	CEB108	94	0.2	Del	Del
D1S172	CEB15	100	0.8	Del	Del
D1S338	CEB88	97	6.3	Del	ND
D1S337	CEB37	87	7.2	Del	ND
D1S173	CEB20	75	7.8	Del	ND
D1F101S2	CEB55	59	10.5	Del	ND
D1S214	AFM147yf8	79	16.4	Del	ND
D1S244	AFM220yf4	78	22.9	Normal	ND
D1S228	AFM196xb4	76	33.2	Normal	ND
D1S234	AFM200yf12	86	57.4	Normal	ND
D1S338	CEB82	31	> 50	Normal	ND

Del: analysis suggests a deletion (see fig 3). ND: not determined.

Discussion

Our case is important for two reasons: first, the phenotypic description is concordant with other cases of 1p deletions thus helping to define the chromosomal region responsible for the abnormalities. Second, because this case was discovered in a sample of patients already used to determine the frequency of subtelomeric rearrangements it allows us to estimate the frequency of such anomalies in patients with idiopathic mental retardation.

To date only eight single case reports and one series of five patients have been reported with deletions of chromosome 1p. These reports have led to the delineation of a syndrome consisting of microcephaly, mental retardation, prominent forehead, deep set eyes, depressed nasal bridge, flat midface, relative prognathism, and abnormal ears. The facial features of the patient described here are consistent with those previously described. Additional clinical features present in the patient have also been reported before: seizures in six of eight patients, minor limb abnormalities in 10 out of 12, and skeletal deformities in five out of seven.[14]

Cytogenetic analyses of previous cases indicated that the region responsible for the phenotype is monosomy for 1p36.33. However, all earlier reports involved additional karyotypic abnormalities, thus making it difficult to relate the phenotype unambiguously to the chromosomal imbalance. Molecular cytogenetic investigation of the patient suggests that the phenotype does indeed arise from monosomy.

Molecular mapping indicates that the deletion is between 16 and 22 cM in length. Although this is a relatively large genetic distance, the fact that the deletion is not cytogenetically visible at the 440-500 band level, together with known expansion of genetic distances towards the ends of chromosomes, suggests that the physical distance is between 5 and 7 Mb.

The deletion in the patient was discovered when our sample of patients with idiopathic MR was surveyed with an HVP from one additional chromosome end. By taking into account the number of probes used and their informativeness, we have estimated the prevalence of cryptic chromosomal abnormalities using equations given by Wilkie.[2] Using our panel of probes, the chance of detecting a random abnormality is 54% for subtelomeric rearrangements; we have discovered four cases out of 99 so the estimate is 7.4%.

Of the four cases of cryptic chromosomal rearrangements so far reported from our sample, two are known to be unbalanced translocations.[1] Molecular cloning of the breakpoint in a third case has shown that the deletion is in fact a truncation of chromosome 22q where telomeric repeats (TTAGGG) have been added directly on to a broken chromosome.[15] Telomeric truncations have previously only been reported for chromosome

16p and it is not known how frequently they occur.[16] Data for the fourth case are not yet conclusive but we have been unable to find any evidence for a translocation. Our data suggest rather that this case too may be a truncation. If so, then telomeric truncations may turn out to be as common as cryptic translocations as a cause of subtelomeric chromosomal rearrangements.

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